REMARKS

Claims 1-6, and 10-19 are pending after entry of the amendments set forth herein. No new matter is added. Reconsideration is requested.

Claim 8 has been rejected under 35 U.S.C. 112, second paragraph. The examiner asserts that the recitation of the term "nucleoside monophosphate" lacks clarity. Applicants respectfully disagree.

As known in the art and defined by traditional chemistry nomenclature, a nucleoside is a purine or pyrimidine base coupled to a ribose or deoxyribose sugar. A nucleoside further linked to a phosphate may be properly termed a nucleoside monophosphate. Nucleotide is an alternative naming convention, but not a required one.

Applicants were not able to find a website corresponding to that cited by the Examiner, but find that the search term "nucleoside monophosphate" in Pubmed produces an abundance of examples, including the widely used textbook by Stryer *et al., Biochemistry* (WH Freeman and Co., fifth edition), which teaches, for example, that "nucleoside monophosphate kinases (NMP kinases), typified by adenylate kinase. These enzymes catalyze the transfer of the terminal phosphoryl group from a nucleoside triphosphate (NTP), usually ATP, to the phosphoryl group on a nucleoside monophosphate"

In view of the above remarks, withdrawal of the rejection is requested.

The Office Action asserts that Claims 1, 3-8, 10-16 and 18 lack novelty over Swartz et al., U.S. Patent no. 6,168,931. Applicants have amended the claims. The present claims recite the use of a cell-free reaction mixture that has been modified by the inclusion of a phosphate free energy source, such as glucose, glutamate, pyruvate, *etc.*, as taught in the specification. Nucleoside triphosphates, which are found in conventional reactions as energy carriers (ATP) or as monomers for the synthesis of mRNA, are replaced with nucleoside monophosphates. Lastly, an exogenous source of phosphate is included.

As described in the specification (see paragraphs 56-62), the standard reaction mixture for a coupled transcription-translation reaction utilizes a phosphorylated energy source, such as phosphoenolpyruvate (PEP). While the use of glucose was previously suggested by Swartz *et al.* (U.S. Patent no. 6,337,191), the synthetic level in such a system was very low.

As shown herein, glucose can be successfully used as an energy source with nucleoside monophosphates when additional phosphate was added to the reaction. Phosphate is

important both for the initial step of glycolysis (glucose to G6P) and for phosphorylation of the NMPs to NTPs. The cell-free reaction was phosphate-limited when using glucose as an energy source. This limitation did not exist in traditional cell-free reactions that typically utilize a phosphorylated energy source such as PEP, creatine phosphate, or even G6P. It was found that experiments using glucose plus phosphate gave protein yields of over 400 μg/mL. Glucose and NMP reactions are beneficial because of the decreased costs and increased stability of these reagents.

Previously, cell-free protein synthesis reactions were limited to using phosphorylated energy sources and nucleoside triphosphates. These compounds are relatively expensive reagents in the cell-free reactions. In addition, the phosphorylated molecules are more susceptible to degradation and create a variable reaction environment with respect to inorganic phosphate concentration. Using glucose and nucleotide monophosphates increases the reaction robustness and homeostasis while also dramatically decreasing costs. The benefits in terms of cost are shown in Table 1:

Table 1: Comparison of cell-free reactions with various energy sources and nucleotides

	NTPs			NMPs (+ phosphate)		
	PEP	G6P	Glucose	PEP	G6P	Glucose
Cost of energy source and nucleotides ($ / mL reaction)	1.88	0.73	0.61	1.28	0.13	0.0108
Typical yields (μg / mL)	700	800	430	700	960	470
Relative product yield (μg protein / $)	1	2.9	1.9	1.5	19.6	**116**

Applicants note that the yield is comparable between the two systems, but the production cost using monophosphate reagents is significantly reduced.

The Examiner has cited Swartz et al., U.S. Patent no. 6,337,191, as anticipatory to the present invention. Applicants note that the present claims have been amended, where claim 1 recites the use of a reaction mixture where there is at least 10 mM of a phosphate-free energy source; nucleoside monophosphates in the absence of exogenous nucleoside triphosphates; and exogenous phosphate at a concentration of at least about 1 mM.

While US 6,337,191 teaches the use of glucose, the patent fails to teach the use of a reaction mixture having the combination of glucose and nucleoside monophosphates in the absence of the nucleoside triphosphates. Applicants respectfully submit the reference fails to teach all the limitations of the claimed invention.

The Examiner has rejected Claims 1 and 3-18 under 35 U.S.C. 103(a) as unpatentable over Swartz et al. U.S. 6,168,931 or Swartz et al. U.S. 6,337,191. Applicants submit that for the reasons set forth above, the presently claimed invention is not made obvious by the cited art.

The Examiner asserts that the direct translation of mRNA to produce proteins does not require nucleoside triphosphates. Applicants note, as discussed above, that phosphate is utilized in glycolysis, and thus the efficient use of glucose as an energy source requires ATP.

The Examiner appears to assert that one can substitute ADP for ATP in a reaction without adverse effect. Applicants note that although the reaction is reversible, it is not thermodynamically equivalent in both directions, and as is well known in the art, energy is required to form ATP from ADP. One cannot run a protein synthesis reaction in the absence ATP, even using pre-formed mRNA templates.

In view of the above amendments and remarks, Applicants submit that the presently claimed invention meets the requirements for 35 U.S.C. 102 and 103. Withdrawal of the rejections is requested.

CONCLUSION

Applicant submits that all of the claims are in condition for allowance, which action is requested. If the Examiner finds that a telephone conference would expedite the prosecution of this application, please telephone the undersigned at the number provided.

The Commissioner is hereby authorized to charge any underpayment of fees associated with this communication, including any necessary fees for extensions of time, or credit any overpayment to Deposit Account No. 50-0815, order number STAN-337.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: December 13, 2010

By: _____
Pamela J. Sherwood, Ph.D.
Registration No. 36,677

BOZICEVIC, FIELD & FRANCIS LLP
1900 University Avenue, Suite 200
East Palo Alto, California 94303
Telephone: (650) 327-3400
Facsimile: (650) 327-3231